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13. ABSTRACT *(Maximum 200*

The purpose of this investigation is to discover the effects of a physical training plus heat acclimation program on exercise performance, thermoregulation, immune function, and reproductive and stress hormone responses in three groups of women: oral contraceptive (OC), Depo-Provera (DP) contraceptive, and eumenorrheic-ovulatory (Eu-Ov) no contraception. Because the number of subjects (n=12) was small in each treatment group (n=1 to 7), few statistical analyses beyond descriptive statistics have been reported. Subjects were stronger, more physically fit, leaner, and heat acclimated at the end of the 8-week physical training program. Reproductive hormone and plasma aldosterone trends showed that subjects responded to the training regimen unremarkably. There was a trend for plasma cortisol levels to decrease (pre-acclimation versus post-acclimation). Immunological measures indicated: (a) no consistent trend in total IgG concentration; (b) OC and DP (combined) appeared to have more lipopolysaccharide antibodies than Eu-Ov; (c) OC and DP (combined) tended to have greater CD-4+ (an immune factor) levels than Eu-Ov; (d) a rising trend in Interferon-gamma (all groups) was observed; and interestingly (e) very low levels of CD4+ and CD8+ were found in all subjects, but the cause is unknown and must be investigated in Year II and Year III.

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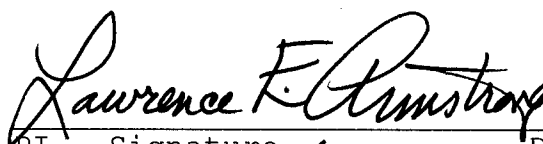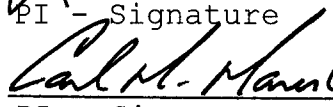
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Lawrence F. Armstrong   10/21/97
PI - Signature   Date

Carl M. Maul   10/21/97
PI - Signature   Date

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Introduction

BACKGROUND

Of the 340,000 women in the Armed Forces of the United States, two-thirds (200,000) serve on active duty and are between the ages of 18 and 30 years. Although they are healthy and active, the unique challenges that military women face (i.e., basic training, physical training, combat) far exceed those in civilian occupations. For example, deployment introduces many unique stressors including harsh environments, primitive housing/sanitary standards, exposure to novel diseases, and close quarters that may affect heat tolerance and immune function.

It is important that an encounter with simultaneous multiple stressors has been recognized as a prominent etiologic factor in military casualties (23,53). For example, concurrent multiple stressors such as a sudden increase in physical training, fever or disease, dehydration, and a lengthy heat exposure were reported during the 5 days prior to exertion heatstroke in 10 soldiers (5).

CONTRACEPTIVE USE BY MILITARY WOMEN

Because military women share quarters with men, stressors related to sexuality arise. These include lack of privacy, a four-to-one ratio of males to females, sexual harassment, and unplanned pregnancy (18). At any point in time, approximately 18,000 active-duty military women are pregnant (61). The majority of these pregnancies are unplanned, in women under the age of 25 (67 %) (25). The need for contraception in the U.S. Armed Forces is authentic, considering individual career advancement, financial resources and mission priorities.

It is difficult to determine accurate statistics regarding the number of military women who use contraceptives (personal communication, LTC Katy Reynolds M.D., Nov 1995). But, it is known that 56% of military women who experience unplanned pregnancy use some form of contraceptive (25). Medical publications (57,59) indicate that 95 % of all sexually active civilian women aged 15 - 44 years, and 74 % of sexually active college females, use some form of contraception in the United States.

A woman's choice of contraceptive method is affected not only by the perceived efficacy and convenience of the technique, but whether additional risks or benefits are associated with its use. While oral estrogen and progestin contraceptive therapies remain the most popular method of pregnancy prevention in the United States, little is known about their effects on exercise performance, thermoregulation, immune function, or reproductive physiology.

The use of long-acting contraceptive methods is increasing in the U.S. Armed Forces because they simplify compliance. For example, Depo-Provera (depot medroxyprogesterone acetate), a long-acting (3-6 months) injectable agent, has an extremely low failure rate (0.0-1.2 per 100 woman-years) and is used by 11 million women in over 90 countries, including the United States (22,58). The U.S. Food and Drug Administration approved its use in 1992, based on WHO epidemiologic data.

Depo-Provera typically provides a three month window of safe and effective contraception, and is ideal for use in military settings (i.e., basic training, deployment, combat). One injection provides a female soldier with three months of uncomplicated birth control. This contraceptive technique is worthy of study because it is used by an ever-increasing percentage of military women and can be administered safely, with little or no supervision, for many years. Although Depo-Provera is the most widely studied injectable steroid formulation (over 500 investigations involving its effectiveness and safety have been published since it became available 29 years ago), very little is known about its effects on exercise performance, thermoregulation, or immune function.

ORAL CONTRACEPTIVES, IMMUNE FUNCTION, AND
RESPONSES TO EXERCISE-HEAT-DEHYDRATION

Thermal balance may be altered by phase of the menstrual cycle, probably due to increased progesterone levels during the luteal phase (40). For example, exercise during the **luteal** phase is characterized by a higher T_{core} (0.4°C) and a higher T_{re} sweat threshold temperature (0.25°C), versus the **follicular** phase (31,40). Although these minor effects have minimal military relevance, they become more important if ambient conditions are hot and humid, and if exercise is intense and prolonged. One study demonstrated, for example, that a 0.6°C T_{re} difference (luteal versus follicular phase) occurred when

women exercised for 60 minutes at 60% VO$_2$max in a 22°C and 60 % rh environment (46). Had the ambient temperature been 35-40°C, the difference between luteal and follicular phase responses would probably have been greater. Admittedly, these minor effects are not as militarily relevant as the effects of oral contraceptives on thermal balance and exercise performance. Although little is understood, it has been shown that oral contraceptives users exhibit more uniform T$_{core}$ and sweating responses than non-users, probably because there is no phasic alteration of **progesterone** levels in these women. Further, injected progesterone (i.e., Depo-Provera) increases basal T$_{core}$ within 24-36 hours of drug administration (31). The impact of these findings on heat tolerance may have significant implications for military women in basic training, deployment, or combat settings and we are examining these issues.

Most military stressors lead eventually to a common response pathway involving activation of the sympathetic nervous system, the secretion of cortisol and epinephrine. The hypothalamic response begins with secretion of corticotropin-releasing hormone (CRH). CRH stimulates secretion of **immunostimulants** (prolactin, LH, and FSH, TSH, growth hormone) and **immunosuppressors** (beta-endorphin, ACTH, cortisol, and alpha-melanocyte stimulating hormone). Plasma cortisol, which is elevated during stress, decreases the levels of antibodies and leukocytes, depresses the ability of white blood cells to digest phagocytized substances, and reduces fever. The degree to which these systems are activated depends on the total stress encountered, the previous physical and mental experiences of the individual, and the degree of control that she or he can exert over the stressful situation (32).

The mechanisms by which these contraceptives affect reproduction are described below (59).
Combined estrogen/progestin formulations: Both estrogen and progestin prevent ovulation by suppression of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secretion. This occurs via inhibition of hypothalamic gonadotropin-releasing hormone (GnRH) release. Levels of LH, FSH, progesterone, and estradiol are suppressed. Besides the inhibition of ovulation, cervical mucus composition is altered and ovum transport/implantation are modified (59).
Depo-Provera: A steroid that prevents follicular maturation, ovulation, and endometrial thickening by inhibiting the secretion of gonadotropins. Depo-Provera is similar in structure to naturally occurring progesterone. Contraceptive plasma levels of this compound are reached within 24 hours of injection and are sustained for 14 weeks after injection (38). Plasma levels of estradiol remain within the normal range (22,38).

Changes in the plasma levels of compounds relevant to reproduction and fertility may alter immune function in women. Elevated **estrogen** levels result in general immunosuppression (8,60) and decreased natural killer cell activity (8). High concentrations of estradiol also serve to suppress immune function in women (41). In contrast, low circulating estrogen is associated with increased total lymphocytes and CD$_4^+$ counts (34). Therefore, **oral contraceptives** (which contain both estrogen and progesterone) should affect the immune system differently from **Depo-Provera** (which acts similarly to progesterone) because various immune cells are affected differentially by estradiol and progesterone (41). Depo-Provera, theoretically, should enhance immune function, in comparison to oral contraceptives. It is not known, however, if such alterations are sufficient to cause illness, or if physical training and heat acclimation will affect specific components of the immune system. Species differences exist; rats and mice exhibit responses that are opposite to those described above for humans (1,42).

HEALTH & PERFORMANCE OF MILITARY WOMEN: RESISTANCE TO INFECTION

A robust immune system (i.e. a high titre of anti-LPS IgG, see below) is desirable for soldiers at all times. Conversely, the combination of overtraining and the stress of new surroundings suppresses immune function and often is blamed for illnesses (i.e. the common cold, sore throat, influenza, mononucleosis) that afflict soldiers and athletes during physical training (52).

The complexities and redundancies of the immune system (16), as well as the many differences in protocols of published studies (62), have contributed to a long-standing polarity of opinion regarding the influence of **acute exercise** and long-term **physical training** on immune function. Although it is known that both women and men show a marked leukocytosis (total white blood cells and polymorphonuclear neutrophils) following exercise of greater than 3 hours duration (63), few definitive conclusions are possible (16,55,62). Similarly, immunologic responses to other stressors inherent in

military training (i.e., **dehydration, heat stress**) have not been investigated adequately in women. This is relevant to the present investigation because military training, especially for new inductees or field units that translocate to stressful environments, subject soldiers to **multiple stressors** in virtually all cases (37). These scenarios and multiple stressors may affect temperature regulation and other vital bodily processes negatively in women (24,45).

SOLDIER HEALTH AND PERFORMANCE: INTESTINAL VIGOR

In addition to immune function, the preceding scenarios illustrate the military relevance of normal nutrient delivery (especially water, salt, and carbohydrates) during physical training (7) and combat (3). However, the research of Gaffin and colleagues (12,15,27,28) and the review of Hubbard et al. (36) have revealed an immunological response to intestinal events, during concurrent multiple stressors, that is not widely appreciated.

During digestion, gram negative bacteria exist in chyme in the small and large intestines. Dead gram negative bacteria provide large amounts of the toxic cell wall component **lipopolysaccharide (LPS)**. LPS found in the outer membrane of gram-negative bacteria are known as **endotoxin**. High levels of plasma LPS seem to be the immediate cause of human septic shock (43). One of the most important discoveries in critical care medicine in the 1980s involved the recognition that LPS may leak out of damaged intestines into the blood, resulting in cardiovascular insufficiency, extensive organ damage or death, in severe cases.

THE PATHOGENESIS OF ENDOTOXEMIA

When LPS enters the portal circulation, one of three fates is possible: (a) detoxification by Kupffer's cells in the liver, (b) inactivation by binding to circulating factors (i.e., HDL, anti-LPS IgG, LPS binding protein, CD-14 or soluble CD-14 receptor), (c) expression of toxicity by binding to LPS binding protein (LBP) and subsequently to CD-14 receptors on the membranes of microphages and other cell types (28). This latter fate results in hypersecretion of **cytokines** (e.g., TNF, IL-1), toxic immune mediators that may cause fever, nausea, vomiting, diarrhea, headache, tissue injury, shock, or death. These symptoms are observed in many cases of heatstroke (35), and have led authorities to suggest that cytokine release is a risk factor for exertional heatstroke (36). Although heatstroke is unheralded and has an unknown etiology in most cases, autopsies of human heatstroke victims have found high titres of plasma LPS and cytokines (13,14). LPS also could be involved in heatstroke by suppressing sweating (9) or cardiac function (44).

Cytokines may alter soldier performance in other ways (23): (a) Both TNF and IL-1 can induce slow wave sleep, suppress appetite (39,54), and cause fever by stimulating prostaglandin E_2 synthesis (21). (b) TNF can induce all features of endotoxin-induced septic shock (43). (c) IL-1 changes the response of arteries to norepinephrine in different vascular beds, and may cause abnormal regional blood flow (47).

EXERCISE-HEAT STRESS, SPLANCHNIC ISCHEMIA, AND LPS

Compared to exercise in cool environments, exercise-heat stress produces a markedly reduced blood flow in splanchnic vascular beds concurrent with an increased heart rate (48). This diversion of blood flow contributes to increased skin blood flow (important for heat dissipation), but carries the threat of compromising the function of splanchnic organs (49,50). This is important because the removal of bacteria and other microorganisms is normally a function of the reticuloendothelial system (RES) in the liver (2). The splanchnic ischemia that accompanies sustained hyperthermia during exercise also has been proposed as a cause of heat exhaustion (6) and the intestinal illnesses seen in 20-30% of all marathon runners (11). If exercise-heat stress or ischemia is great, an increase in plasma LPS may occur due to increased gut permeability. This phenomenon has been observed in primates, cats, miniswine, and rats (28,51). These animals demonstrated that core **hyperthermia** must reach severe levels (42 - 45°C) before lethal increases in LPS occur (28,29,36).

Other stressors enhance the entry of gut-derived LPS into the circulation: hypovolemia, splanchnic artery occlusion, and diarrhea (28,36). Hypoxia also has been shown to potentiate the production of TNF and IL-1 in human blood mononuclear cells, after resting exposure to subthreshold levels of LPS (30).

Human studies suggest that **exercise and/or physical training** play an important role in endotoxemia. For example, plasma LPS levels were elevated after strenuous exercise by triathletes and ultramarathon (89.5 km) competitors: (a) Bosenberg et al. (12) found that LPS rose and the "natural" anti-LPS IgG (the antibody formed in response to LPS) decreased during competition; (b) Brock-Utne et al. (15) observed that 80 % of collapsed runners had elevated levels of plasma LPS. The casualties with low/normal levels of LPS, but high levels of anti-LPS IgG, symptoms were far less severe than those with high/abnormal plasma LPS, and low levels of anti-LPS IgG; this latter group required two days to recover. Thus, the presence of a higher titre of anti-LPS appeared to protect the runners, possibly because they had been autoimmunized during daily training.

A critical question has emerged from these human studies. Can the level of "natural" anti-LPS antibodies be manipulated in soldiers to effectively reduce susceptibility to heat illness? The hypothetical answer suggests that part of the benefit of **physical training** for soldiers might be to increase the natural plasma levels of anti-LPS IgG. This could occur as small amounts of LPS enter the circulation, during strenuous training, on a daily basis. Because LPS stimulates a hypersecretion of the cytokines TNF and IL-1, this issue has great military relevance because cytokines may increase casualty rates (see above) and the susceptibility to heat illness (36).

Purpose

It is important that an encounter with simultaneous **multiple stressors** has been recognized as a prominent etiologic factor in military casualties (23,53). The goal of this study is to provide important information to reduce complications associated with stressful environments and therefore decrease casualties in military women. Comprehensive information about the health of military women facing multiple stressors is not currently available. Our study has been designed to clarify the ability of exercise training and heat acclimation to minimize the effects of multiple stressors on (a) exercise responses in the heat while dehydrated, (b) immunocompetence, and (c) hormone levels.

It is essential to the goals of this research project that we meticulously control the onset of testing and training for each subject's menstrual phase and status, and document compliance to contraceptive therapies.

Because the effects of oral and injectable contraceptives on physical training and heat acclimation are virtually unknown, and because the immune system maintains a constant state of personal health by interacting with every organ system in the body, the following technical objectives and hypotheses have great relevance to military women and military units.

Technical Objectives

A. Primary Longitudinal Objectives

1. To evaluate differences among the three groups with respect to immune function before and after an eight-week training/heat acclimation program.

2. To evaluate differences among the three groups with respect to the exercise-heat tolerance test (EHT) responses before and after an eight-week training/heat acclimation program.

3. To evaluate differences among the three groups with respect to reproductive hormone status before and after an eight-week training/heat acclimation program.

B. Secondary Longitudinal Objectives

1. To evaluate differences among the three groups with respect to stress hormones before and after an eight-week training/heat acclimation program.

2. To evaluate differences among the three groups with respect to body composition and VO_2max before and after an eight-week training/heat acclimation program.

C. Dependent Variables: Categorical Definitions

1. Reproductive hormone - estradiol, progesterone, sex hormone binding globulin

2. Immune function - CD-4+, CD-8+, anti-LPS IgG, total IgG, HSP_{70}, IL-10, IFNg
     (abbreviations defined on page 13)

3. Exercise-heat tolerance -
    a) thermoregulatory markers: rectal temperature, skin temperature, whole body & local sweat rate, skin blood flow
    b) fluid-electrolyte balance: aldosterone, osmolality, hematocrit, hemoglobin, plasma volume shift
    c) exercise performance: heart rate, blood pressure, exercise tolerance time, rating of perceived exertion, rectal temperature, glucose, lactate

4. Stress hormones - cortisol, epinephrine, norepinephrine

D. Independent Variables:

A. groups: oral contraceptive users (ORAL), Depo Provera users (DP), eumenorrheic ovulatory women taking no form of birth control (EU-OV)

B. time: pre-training/heat acclimation
      post-training/heat acclimation

Null Hypotheses

A.   Null Hypotheses Associated with Primary Longitudinal Objectives

1.   There will be no significant differences among the three groups with respect to immune function before and after an eight-week training/heat acclimation program. We expect that the Depo-Provera group will exhibit the most favorable immune response.

2.   There will be no significant differences among the three groups with respect to the exercise-heat tolerance test (EHT) responses before and after an eight-week training/heat acclimation program. We expect that the Oral Contraceptive group will exhibit the most favorable thermoregulatory response.

3.   There will be no significant differences among the three groups with respect to reproductive hormone status responses before and after an eight-week training/heat acclimation program. We expect that the EU-OV group will exhibit the greatest perturbations in reproductive hormone status.

B.   Null Hypotheses Associated with Secondary Longitudinal Objectives

1.   There will be no significant differences among the three groups with respect to stress hormones before and after an eight-week training/heat acclimation program. We expect that the magnitude of the changes in stress hormone levels will be equivalent among the groups.

2.   There will be no significant differences among the three groups with respect to body composition and maximal aerobic power (VO$_2$max) before and after an eight-week training/heat acclimation program. We expect that the changes in body composition and VO$_2$max will be equivalent among the groups.

Statement of Work / Experimental Scope

Technical Objective: To evaluate the effects of oral and injectable contraceptives on hormones (i.e., reproductive, fluid-electrolyte, stress), immune system function, and exercise-heat-dehydration tolerance.

Task 1:   Months 1 - 4: Order supplies, materials; prepare equipment and chamber. Oversee graduate students/ technicians and budgetary matters. Insure that research meets regulations of Environmental Health & Safety and the Institutional Review Board for Human Subjects.

Task 2:   Months 4 - 7: Recruit, screen, identify, and brief test subjects. Conduct preliminary screening to eliminate subjects with exclusionary criteria.

Task 3:   Month 8: Conduct Intensive screening of subjects. Select >15 subjects in three groups: oral contraceptive users, Depo-Provera users, and EU-OV. Collect descriptive subject data.

Task 4:   Month 8: Collect two baseline blood measurements of immune system markers and hormones (reproductive, fluid-electrolyte), verify normalcy of reproductive function, and verify menstrual phase timing.

Task 5:   Month 8: Prepare environmental chamber and instruments for testing. Conduct >15 exercise-heat tolerance tests (90 min each) at 38°C, to document pre-training responses.

Task 6:   Months 8 - 10: Conduct eight-week training program for >15 women (6 days/week; 3 days involve heat exposure up to 90 min).

Task 7:   Months 8 - 10: Collect blood samples throughout training period, to evaluate hormones (reproductive, fluid-electrolyte), immune system function, normalcy of reproductive function, and menstrual phase timing.

Task 8:	Month 10: Prepare environmental chamber and instruments for testing. Conduct >15 exercise-heat tolerance tests (90 min each) at 38°C, to evaluate post-training responses. Collect blood samples to identify post-training levels of hormones, immune markers, and timing of menstrual phases.
Task 9:	Months 10 - 12: Laboratory analyses. Enter data into spread sheet. Submit annual report when required.
Tasks 10 - 17:	Months 13 - 24: Repeat Tasks 1 - 8 above, to begin collecting data on >15 additional women, bringing the number to 30 total subjects.
Tasks 16 - 25:	Months 25 - 36: Repeat Tasks 1 - 8 above, to begin collecting data on >15 additional women (goal: bring the number to 15 in each treatment group).
Task 26:	Submit final report when required.
Task 27:	Prepare abstracts for submission to scientific conferences. Prepare manuscripts for submission to journals for publication.

Experimental Methods and Procedures

This section presents the experimental design and procedures which have been used to meet study objectives. In order to achieve the necessary number of subjects (n = 15) for each group, we are performing all screening procedures, and training the three different groups, in multiple iterations over three years using a balanced distribution of subjects to control for variance.

SUBJECT CHARACTERISTICS

Female civilian students attending the University of Connecticut were recruited by announcements posted on bulletin boards, in classrooms, and the daily campus newspaper. The University of Connecticut has approximately 8,000 female students within the 18-34 year age range, and we are experienced in recruiting women for experimental and training studies. University staff and women living in adjacent communities also were recruited. Potential volunteers were given a description of the objectives, procedures, risks, and time commitments required for the study. All subjects were asked to provide written voluntary consent to participate, in compliance with the Institutional Review Board for Human Subjects at The University of Connecticut. Interested subjects completed a medical history and physical activity questionnaires, and were interviewed by one of the investigators.

All subjects are required to meet the following criteria:
a) aged 18 to 34 years; b) within the average range ($\pm$ 2 SD) of U.S. military women for height (162 $\pm$ 13 cm) and weight (60 $\pm$ 16 kg); c) in good health, as determined by a medical and gynecological examination (private physician, within the previous 12 months) including a normal Papanicolaou smear; d) free of any chronic disease including thyroid disease and hyperprolactinemia; e) lack of any recent (within three months) changes in menstrual status; f) appropriate activity history; g) no history of eating disorder or depressive illness within the past three years and an appropriate score on the Eating Disorders Inventory (EDI); h) the absence of any contraindications revealed in a medical history that might preclude participation in the study, including a history of heat-related illness, endotoxemia, chronic respiratory disorder, cardiovascular disease, hypertension, metabolic disorders, convulsive disorders, drug or alcohol dependence; and i) not routinely taking a prescription or over-the-counter medication that would alter variables measured herein. All subjects must be non-pregnant, for the duration of screening and testing, as determined by blood sample analysis for HCGH. All University of Connecticut students are required to have current inoculations.

Subjects were asked to report any gastrointestinal or respiratory tract illnesses, or superficial injuries (i.e. abrasions, cuts) incurred during their involvement in the project. All subjects were performing no more than 90 minutes of aerobic activity per week for the previous 12 months, and had a maximal oxygen uptake (VO_2 max) less than 42 ml$\cdot$min$^{-1}\cdot$kg^{-1}. Subjects were paid for their participation.

INCLUSION OF MINORITY TEST SUBJECTS

Women representing minority groups have been encouraged to participate because nothing is known about racial differences in these responses. Their responses will be compared to non-minority subjects, in **post hoc** statistical analyses. The student body at the University of Connecticut includes 12 % minority representation. In fact, two graduate students in our doctoral program are women of African-American descent and will be involved in this project for its entire duration.

EXPERIMENTAL DESIGN

To test the various hypotheses set forth in this investigation, we are utilizing three groups of women: a) females currently ingesting an oral contraceptive (ORAL) for a minimum of three months prior to the study (n = 15), b) females receiving depot medroxyprogesterone acetate (DP; Depo-Provera) long-acting contraceptive therapy for a minimum of three months prior to the study (n = 15), and c) eumenorrheic ovulatory (EU-OV) females (n = 15).

During the Fall semester of academic year 1996-97, subjects were recruited and underwent a two month (during the months of October, November and December) preliminary screening procedure. Beginning in January, subjects performed an intensive one-month screening procedure, followed by an 8-week exercise training plus heat acclimation program. Exercise training sessions were held six days per week, with the heat acclimation sessions (alternate days, up to 90 min·day^{-1}) comprising three of those days. Maximal oxygen uptake tests and exercise-heat tolerance (EHT) tests were performed prior to, and at the end of the 8-week training program. It is our contention that a non-training control group is not necessary for this study for two reasons: a) because it represents a scenario with little or no military relevance and b) the pre- and post-training measurements for all groups in this study allow us to compare untrained and trained states.

Weight, menstrual patterns, nutritional habits, training habits, and any atypical stressors were monitored throughout the study. Subjects were weighed (kg) during all laboratory visits. Menstrual bleeding patterns and exercise reports were monitored daily via diary and training cards. Subjects recorded any medications that they were consuming in their menstrual diary. Seven day nutritional dietary records were completed during the first seven days of each menstrual cycle (or 28 day period) to make sure that dietary intakes are appropriate to support the nutritional demands and caloric expenditure of training. Thus, any significant changes in dietary habit were documented as thoroughly as possible. Each subject's health status is of great concern to us, from an ethical perspective, and to ensure that she will continue with the training program. The Women's Health Center on our campus is available for appointments and to collect clinical data concerning illness.

MENSTRUAL CYCLE AND COMPLIANCE MONITORING

All eumenorrheic ovulatory (EU-OV) subjects were asked to participate in menstrual screening procedures that accurately determine their ovulatory status and the length of their luteal phase. These were determined via blood samples that pinpoint the onset of the luteinizing hormone (LH) surge to within 12-24 hours. Subjects were asked to maintain menstrual logs to document menstrual cycle length and duration of menstrual flow days prospectively. During the menstrual cycle immediately preceding training, and during the second month of physical training, all eumenorrheic subjects had blood sampled during days 2, 3 or 4 (until 1 day after the peak LH concentration had been reached), one blood sample taken 7 days later, and one sample taken on day 23, to document menstrual phases. These blood samples were analyzed on the same day for estradiol (E_2), progesterone (P_4), luteinizing hormone (LH), follicle stimulating hormone (FSH), and sex hormone binding globulin (SHBG).

All subjects ingesting oral contraceptives (ORAL) were asked to report the exact preparation, duration of use and compliance to therapy. All oral preparations must be of the ethinyl estradiol type (over 25 commercial products exist). Women ingesting preparations that include mestranol were excluded from the study. Subjects were asked to provide empty pill packs to the investigators to document therapy information. During the cycle immediately preceding training, and during the two months of training, all ORAL users had blood sampled during day 2, 3, 4, or 5 following the onset of menses to document compliance to therapy. These blood samples were analyzed for E_2, P_4, and SHBG.

All subjects receiving long-acting Depo-Provera (DP) contraceptive therapy were asked to report the exact dose, preparation, and duration of use. During the cycle immediately preceding training, and during the two months of training, all injectable DP users had blood sampled during days 2, 3, 4, or 5 of a given 28 day period (initiated on an arbitrary day) to document therapy. These blood samples were analyzed for medroxyprogesterone acetate (i.e., provided in the contraceptive DP), E_2, P_4, and SHBG.

BODY COMPOSITION AND MAXIMAL OXYGEN UPTAKE MEASUREMENTS

Body composition analyses were performed during the first seven days of each menstrual cycle or 28 day period, and at the beginning and end of the training period. Body density was determined from underwater weighing. Percent body fat and lean body mass were calculated according to Siri (56). All subjects completed an incremental run to exhaustion (modification of Costill and Fox protocol) on a motorized treadmill for determination of VO_2 max (17). These tests were performed during the intensive screening period and following the exercise training program. Briefly, subjects ran at an appropriate speed for four minutes at 0% grade. After four minutes, the grade was increased to 4% for two minutes. The grade was then be increased 2% every two minutes until the subject reached volitional exhaustion. Two of the three following criteria were used to verify the attainment of VO_2 max: 1) no further increase in VO_2 (less than 150 ml•min^{-1}) with an increase in grade, 2) heart rate greater than 90% of predicted maximum (220 minus age), and 3) respiratory exchange ratio greater than 1.1.

EXERCISE-HEAT TOLERANCE TESTING

Exercise-heat tolerance (EHT) tests were performed at the beginning and end of the 8-week training program. To enhance the stress associated with the EHT, subjects undertook a 24-hour water restriction prior to testing, providing an approximate -3% level of dehydration. The EHT involved walking on a motorized treadmill at 93.6 m•min^{-1} and 5% grade (4). Walking speed was verified for each test with a hand-held tachometer (Model 8240-20 Cole Parmer Instrument Co., Chicago, IL). The mean temperature and % humidity were 38°C and 50-70%, respectively. Air flow was 2.3 m•s^{-1}. No water was consumed during the EHT. The test was terminated if: a) T_{re} reached 39.5°C, b) the heart rate exceeded 180 beats min^{-1} for five consecutive minutes, c) the subject showed signs of heat illness, d) the subject asks to stop, or e) she completed 90 minutes of exercise.

A schematic representation of events during each EHT test appears as **Figure 1**. The following physiological and perceptual measures were taken at regular intervals before, during and after EHT testing: oxygen uptake, minute ventilation, and respiratory exchange ratio using an on-line system (Medical Graphics Corporation); whole-body sweat rate (± 50 g) via body weight differences; mean weighted skin temperature (4 sites) via infrared temperature scanner (Ototemp, Inc.); subjective ratings of perceived exertion (10); and thermal stress (64). Rectal temperature (rectal thermistor, YSI Inc.), heart rate via cardiotachometer (Polar Electro), and exercise time were the primary variables representing exercise-heat tolerance. Measurements of local chest sweat rate using resistance hygrometry (Model B1-102, Bi-Tronics, Inc.) and local skin blood flow via laser doppler flowmeter (Techtronics, Inc) will begin in Year II and continue through Year III (see section below titled, "Recommendations Regarding the Statement of Work" item 2).

EHT TESTING: MENSTRUAL PHASE AND CONTRACEPTIVE THERAPY

All eumenorrheic ovulatory women were tested during day 2, 3, 4, or 5 of their menstrual cycle (i.e., early follicular phase). All oral contraceptive users were tested on day 2, 3, 4, or 5 of the 7 day placebo period for their respective pill packs. All Depo-Provera users were tested on day 2, 3, 4, or 5 of a 28 day period arbitrarily initiated during the preliminary screening period. The specific day on which testing occurs remained consistent for each subject.

EXERCISE TRAINING PLUS HEAT ACCLIMATION

The exercise training program lasted 7-8 weeks--two menstrual cycles--in duration. It was necessary to admit subjects into the training program in a staggered fashion to account for timing differences in menstrual cycle phase and contraceptive therapy. Training sessions were held six days per week (Monday - Saturday). Training sessions on Tuesday, Thursday, and Saturday involved strenuous

group running and calisthenics (push-ups and sit-ups), with a progressive increase in volume and speed of running across weeks. The number of push-ups and sit-ups also progressively increased for eight weeks. All of these training sessions were supervised. Training sessions on Monday, Wednesday, and Friday also were supervised, and involved exercise-heat exposures (environmental chamber, 36°C, 50-70 % rh) progressing toward 90 minutes of continuous exercise-heat acclimation each day. These sessions entailed 5-6 subjects exercising at one time, employing a circuit of bench stepping, cycle ergometry, and treadmill walking. Subjects were permitted to drink water ad libitum during these sessions. Subjects were encouraged to exercise continuously as long as possible during these sessions, but were asked to remain in the chamber even if they stop exercising, for the complete 90 minute period. However, subjects were removed from the environmental chamber if: a) T_{re} reached 39.5°C, b) heart rate exceeded 180 beats·min^{-1} for 5 consecutive minutes, or c) the subject showed signs of heat illness.

RESTING BLOOD COLLECTIONS: HORMONE & IMMUNE SYSTEM ANALYSES

Figure 2 presents the timeline for resting hormone and immunological analyses during the preliminary screening, the intensive screening, and the 8-week heat acclimation/training program. Blood samples were obtained by needle and syringe or indwelling cannula, collected into serum or plasma collection tubes and then processed, centrifuged, stored when appropriate at -80°C, and analyzed.

Concerning reproductive hormones and aldosterone, these blood samples allowed two baseline measurements (with respect to menstrual phase) prior to the start of the training protocol. Regarding the immune factor measures, two baseline measurements of each blood variable also were made prior to the start of the training protocol.

EHT BLOOD COLLECTIONS: HORMONE AND IMMUNE SYSTEM ANALYSES

The EHT tests were conducted before and after the 8-week training program, and on the day following the resting blood collections described above. Pre-exercise and immediate post-exercise blood samples were obtained via indwelling cannula and analyzed for whole blood, plasma, or serum concentrations of cortisol, epinephrine, norepinephrine, lactate, glucose, osmolality, hematocrit, hemoglobin, anti-LPS, and IgG. Analyses of anti-LPS and Total IgG also were performed on 24-hour and 48-hour post-exercise blood samples

REPRODUCTIVE HORMONE AND IMMUNE SYSTEM MEASUREMENTS

Serum estradiol, progesterone, prolactin, TSH, FSH, LH, thyroxin and SHBG were analyzed in the Department of Fertility and Reproductive Endocrinology at New Britain General Hospital via immunoassay (Immulite). This procedure provides excellent sensitivity and reliability by combining highly specific antibodies with enzyme-amplified chemiluminescent chemistry and a proprietary wash technique. Serum aldosterone (19) and cortisol (33) concentrations were analyzed in the Human Performance Laboratory (University of Connecticut) via radioimmunoassay.

CD4 and CD8 determinations were performed on unseparated cells in whole peripheral blood, at the U.S. Army Research Institute of Environmental Medicine (USARIEM), Natick, MA. Briefly, whole heparinized blood was incubated with the specific antibody followed by a fluorescent second antibody. Red blood cells were lysed, the white cells fixed with 1% paraformaldehyde and the samples analyzed by flow cytometry.

IL-10, TNF, and INF-g were measured at USARIEM in plasma by commercially available ELISA kits. CD-14 and CD-45 were also measured, but only to provide data necessary to properly determine CD-4 and CD-8. Anti-LPS IgG was measured by an ELISA produced in-house, and Total IgG by an automated immunoenzyme system (Monarch) at USARIEM (26). We will attempt to measure HSP$_{70}$ via either Western blot or PCR technology at USARIEM in Years II and III. Plasma norepinephrine and epinephrine levels (Year I) were analyzed via an HPLC technique (33).

Hematocrit was determined by microcapillary technique. Hemoglobin was measured by the cyanmethemoglobin method (Kit 525, Sigma Chemical, Inc.) Percent change in plasma volume was calculated using hematocrit and hemoglobin values (20). Osmolality was measured by freezing point depression (model 5004 micro-osmette, Precision Systems, Inc.). Plasma lactate and glucose values were determined using a model 2300 glucose and L-lactate analyzer (Yellow Springs Instruments).

Abbreviations Used in Figures 1 and 2

Figure 1

Dehyd. = dehydration to -3 % of body weight
HTT (also EHT) = the exercise-heat tolerance test
        conducted in our environmental
        chamber (38°C, 50-70% rh)
V_E = minute ventilation (L/min)
VO_2 = oxygen consumption
RER = respiratory exchange ratio (CO_2/O_2)
BP = blood pressure (systolic/diastolic)
MWST = mean weighted skin temperature,
        taken at four sites
T_{re} = rectal temperature

Figure 2

ALD = aldosterone
CD_x = cluster of differentiation (x = 4,8,14,45)
DP = Depo-Provera subjects
E_2 = estradiol
EE = ethinyl estradiol
EU-OV = eumenorrheic ovulatory subjects
F = follicular phase
FSH = follicle stimulating hormone
HSP_{70} = heat shock protein
IFN-g = interferon N
IG-1 = immunoglobulin 1
IL-x = interleukin (x = 6,10)
L = luteal phase
LH = luteinizing hormone
MPA = medroxyprogesterone acetate
ORAL = oral contraceptive subjects
P_4 = progesterone
PRL = prolactin
SHBG = sex hormone binding globulin
T_4 = thyroxin
TSH = thyroid stimulating hormone

FIGURE 1

Heat Tolerance Test Schematic

| Dehyd. | Subject set-up | Heat Equilibration Standing | Exercise heat tolerance test |

Time (min): -20 0 3 7 15 20 30 40 45 60 75 90 95 or termination
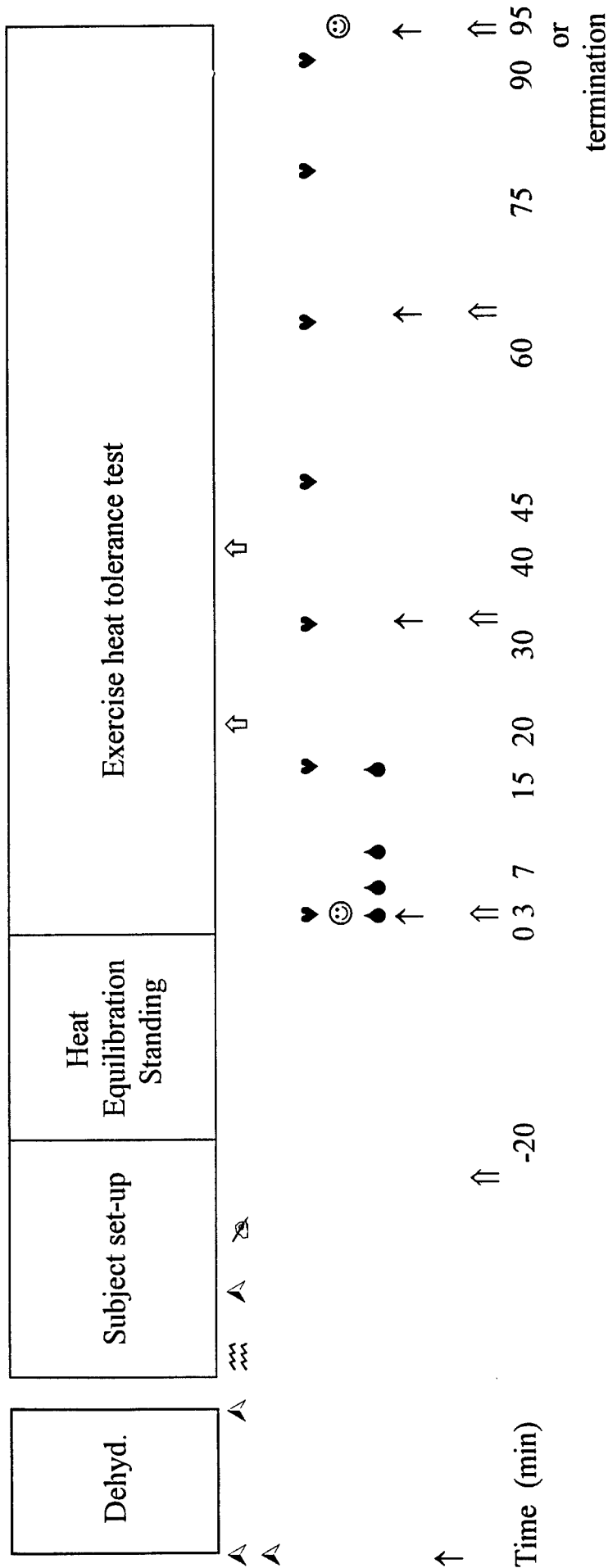
Measurements

$\wr\wr\wr$ - void bladder and bowel; weigh exercise clothes prior to dressing and following final body weight

- rectal probe and cannula (insert prior to pre HTT & remove prior to post body weight)

☺ - blood draw @ following 20 min standing heat equilibration and at termination

⇧ - VO_2, V_E, RER @ min 20 & 40

● - Local chest sweat rate (Dew point sensor) @ min 0, 3, 7 & 15

➤ - HR, T_{re}, RPE, Thermal stress, @ min 0, every 15 min & at 89 min or at termination

⋏ - Body weight @ pre & post dehydration, pre & post HTT

↑ - BP @ min 0, 30, 60 & 90 or termination & 5 min post

⇑ - Skin blood flow & MWST (OtoTemp) @ min -25, 0, 30, 60, & 90 or termination

Preliminary Screening   Intensive Screening   8 Week Training Program

| November | December | F(period 1) January | F(period 2) February | F(period 3) March | April |

F OV L F OV L   F OV L   F(period 1) OV L   F(period 2) OV L   F(period 3) OV L   F(period 4)

0 wk   8 wk

PRL
TSH
T_4

EU-OV
ORAL
DP
(8 ml)

E_2
P_4
LH
FSH
SHBG

E_2
P_4
LH
FSH
SHBG

EU-OV
(6-7 times x 3ml; 15-21)

CD_4
CD_8
CD_14
CD_45
IG-1
HSP_70
IL-10
IL-6

E_2
P_4
ALD
SHBG

EU-OV
ORAL
DP
(22 ml)

ORAL: EE (2 ml)
DP: MPA (2 ml)

ORAL: EE (2 ml)
DP: MPA (2 ml)
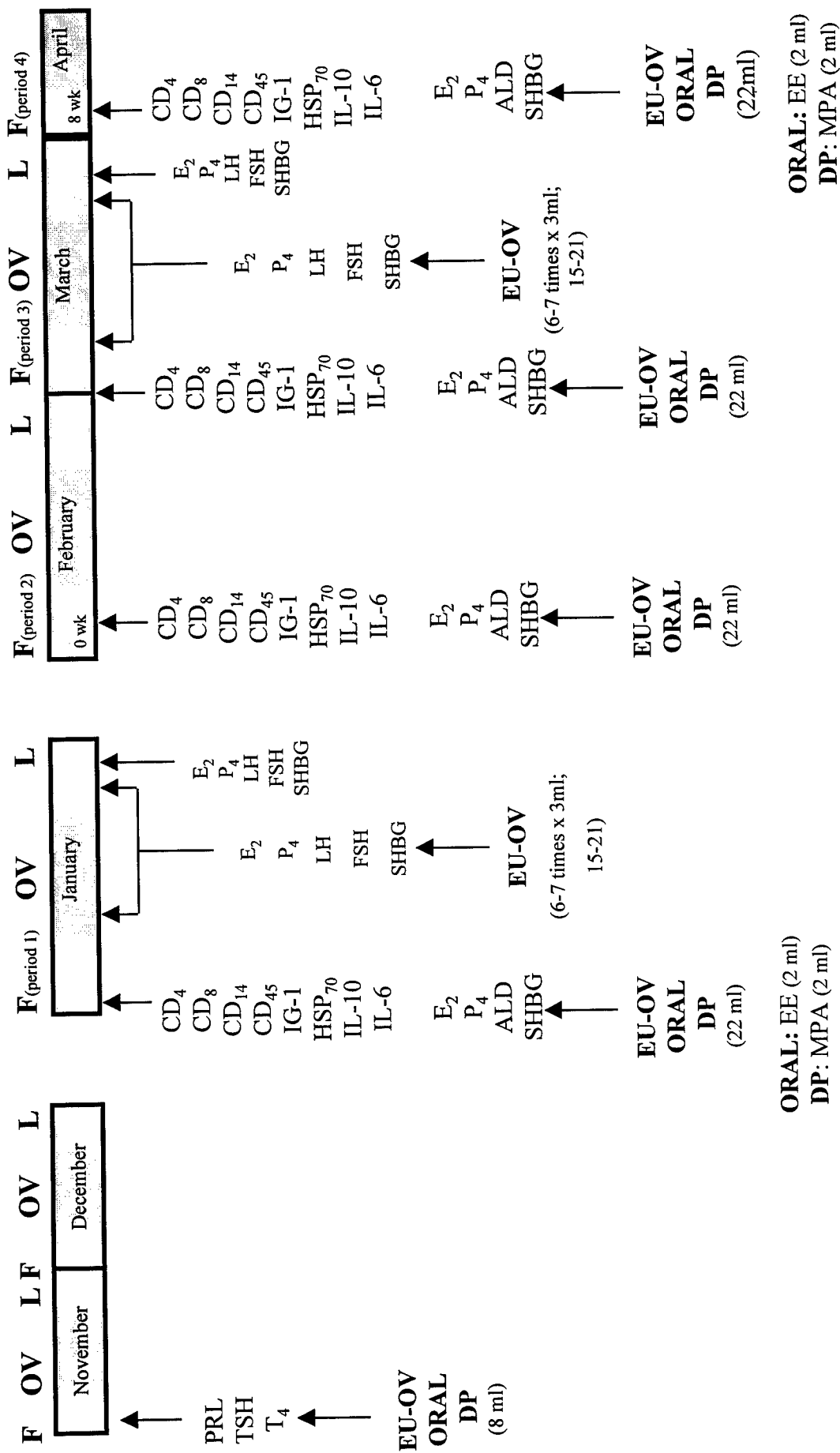
FIGURE 2: Resting blood collection for hormone and immune system measures. (Volumes of blood samples appear at each time point).

STATISTICAL METHODS

In Year II and Year III, data will be entered into an IBM computer on the CSS:Statistica™[3.1], Statsoft Data Management System. We will use common descriptive statistics to describe the data sets. In addition, a wide range of multivariate statistics will be used to determine group differences, main effects, interactions and relationships between variables. Appropriate post hoc tests will be performed where significant F ratios are found. Where appropriate (e.g. hormonal response curves), data will also be analyzed by assessing the area under the curve response (AUC) as calculated by the trapezoidal method, after the baseline has been subtracted. Analysis of variance will then be performed on the square root of the AUC. When appropriate, non-parametric analyses also will be used. A significance level of 0.05 will be used to detect significant differences.

Results and Discussion

YEAR I DATA PRESENTATION

Because the number of subjects in each experimental group was small (n = 1 to 7) during the initial year of testing, most data below are presented as group means, and no between-group statistical comparisons (i.e., analyses of variance) were attempted. The number of subjects in each treatment group will be increased, as originally proposed, during Year II and Year III of this investigation. Analysis of variance will be performed when valid (based on the n in each group) for the Year II annual report, but definitely will be presented in the Final Report of Year III.

Because only one of the 12 test subjects was using Depo Provera (DP), special efforts are being made during Fall, 1997 to recruit DP users on the University of Connecticut campus and in the surrounding community, for testing in Year II.

The personal characteristics of the twelve test subjects were as follows (mean ± SE): age, 21 ± 1 yr; height, 64.9 ± 0.7 cm; body mass, 66.64 ± 2.63 kg; maximal aerobic power, 37.1 ± 1.0 ml O_2/kg/min.

No serious illnesses, serious injuries, or untoward events occurred during Year I of testing.

REPRODUCTIVE HORMONES: SCREENING & EFFECTS OF 8 WEEKS OF TRAINING

The 12 women who participated in the study represented eumenorrheic women (n=4), oral contraceptive users (n=7) and Depo-Provera users (n=1). All subjects who participated in this investigation passed a hormonal assessment during the screening process. The initial assessment consisted of normal baseline TSH, free T_4, and prolactin. Data for the eumenorrheic group are presented in terms of menstrual cycle milestones, while data for the oral contraceptive group are presented in terms of hormonal assessment, measured during the placebo week of each subject's pill pack. The data of the single subject using Depo Provera has been included in the oral contraceptive group, for this Year I Annual Report.

Subjects in the eumenorrheic group were assessed for one complete menstrual cycle; this assessment consisted of E_2, LH, FSH, SHBG, prolactin and P_4 measurements. During the menstrual cycle assessments, blood was drawn during the early follicular phase (days 2-6), the mid-to-late follicular phase (days 8-19), and the mid-luteal phase (5-7 days following the LH peak). Cycle length, ovulation day, normal luteal phase, normal hormonal characteristics and previous cycle lengths were utilized as criteria for admission to the study. Once admitted to the study, eumenorrheic subjects underwent a second complete menstrual cycle assessment during the second month of the 8-week training program.

The oral contraceptive group was evaluated for these same hormones, during the training sessions and following the 8 week training program. Oral contraceptive users had hormonal assessments (estradiol, progesterone, prolactin, SHBG) done during the placebo phase of their pill regimen.

Table 1 shows that, overall, study participants responded to the training regimen unremarkably. The ovulatory status of the eumenorrheic group at post-training appears similar to the pre-training period. Cycle length, follicular phase length, and luteal length appear to have remained unchanged. Hormonal responses of the contraceptive users also appear to be unremarkable.

Table 1 - Reproductive Hormones: Pre- and Post-Training

Eumenorrheic Group	Pre-Training	Post-Training
Early Follicular		
Estradiol	32.7±5.5	49.5±0.0
Progesterone	0.8±0.2	0.7±0.0
Prolactin	21.4±4.3	12.4±0.0
SHBG	31.5±6.3	44.4±0.0
Mid-Cycle		
Peak estradiol	236.8±35.8	350.5±21.5
Peak LH	32.3±2.4	34.2±1.2
Mid-luteal		
Peak estradiol	115.4±35.5	168.0±22.0
Peak progesterone	9.9±1.4	11.3±0.3
Cycle Parameters		
Cycle length	29.0±1.3	29.5±0.5
Ovulation day	17.0±0.4	17.5±0.5
Follicular length	17.0±0.4	17.5±0.5
Luteal length	11.8±0.9	12.0±0.0

Contraceptive Group	Pre-Training	Post-Training
Estradiol	38.3±8.5	32.5±5.3
Progesterone	0.5±0.1	0.7±0.1
Prolactin	14.5±2.5	13.9±4.8
SHBG	139.5±21.5	189.4±33.4

All values are Means ± SE.
Abbreviations:
LH - luteinizing hormone
SHBG - sex hormone binding globulin

During the periods shown in **Table 1**, mean (± SE) resting aldosterone levels (i.e., not associated with exercise or heat exposure) were not significantly different at the pre-training (638.3 ± 109.5 pmol/L) and post-training (730.3 ± 96.9 pmol/L) time points.

PHYSICAL TRAINING AND HEAT ACCLIMATION (8 WEEKS)

The proposed 8-week training schedule was accomplished with a very high compliance rate for daily exercise sessions and heat exposures (>98% including illness). Three days of each week were spent performing stretching, calisthenics (i.e., pushups and situps), and walking/running a 2.8-mi course around campus. The remaining three training days were spent in the environmental chamber, performing various types of exercise (i.e., cycling, treadmill walking, bench stepping) in conditions of 36°C, 50-70 % rh; these sessions were designed to induce heat acclimation in all subjects. During exercise-heat acclimation sessions, subjects exercised 77-82 min out of the total 90 min heat exposure. Subjects did not train one day per week.

The following measurements were made to track the progress of the physical training of test subjects, including:

-- number of pushups and situps (i.e., abdominal crunches) completed in 1 min
-- time to complete the 2.8 mi outdoor course
-- body composition changes (i.e., body mass, % body fat, fat-free mass)
-- maximal aerobic power (VO_{2max}).

The physical training criterion variables appear in **Table 2** below, as recorded during the initial and final weeks of this training program. Values reflect group means ± SE. Column four indicates which values were significantly different (pre- versus post-acclimation), as assessed by paired t-tests:

TABLE 2 (All subjects combined, n = 12).

MEASUREMENT (units)	INITIAL WEEK *	FINAL WEEK	P>.05
pushups (per 60 sec)	13 ± 2	29 ± 4	yes
situps (per 60 sec)	47 ± 5	68 ± 5	yes
2.8 mi run time (min)	44 ± 1	31 ± 1	yes
body mass (kg)	66.6 ± 2.6	66.4 ± 2.4	no
body fat (%) **	28.7 ± 1.3	27.1 ± 1.3	yes
fat-free mass (kg) **	47.0 ± 1.2	48.0 ± 1.3	yes
VO_{2max} (L)	2.45 ± 0.06	2.67 ± 0.47	yes
VO_{2max} (ml/kg/min)	37.1 ± 1.0	40.7 ± 0.1	yes

Abbreviation: VO_{2max} - maximal aerobic power
* - pre-training
** - hydrostatic weighing

These measurements indicate that the 12 test subjects were stronger, more physically fit, and leaner at the end of the 8-week physical training program.

EXERCISE-HEAT TOLERANCE (EHT) TESTING

Specialized EHT tests were administered to each subject, before and after the 8-week training period. EHT tests consisted of walking on a motorized treadmill at 93.6 m·min⁻¹ and 5% grade. The mean temperature and relative humidity were 38°C and 50-70%, with an air flow of 2.3 m·s⁻¹. To enhance the stress associated with the EHT, subjects underwent exercise combined with a period of 24-hour water restriction prior to testing, providing approximately -2.9% level of dehydration during the EHT. No water was consumed during the EHT.

The values recorded during the pre-training and post-training EHT tests appear below in **Table 3**, expressed as group means ± SE. Column four indicates which values were significantly different (pre-versus post-acclimation), as assessed by paired t-tests:

TABLE 3 (All subjects combined, n = 12).

MEASUREMENT (units)	PRE-ACCLIMATION	POST-ACCLIMATION	P>.05
pre-exercise dehydration (% loss)	- 2.9 ± 0.2	- 2.9 ± 0.1	no
exercise time to exhaustion *	35.4 ± 4.5	67.7 ± 5.4	yes
final heart rate (beats/min) **	183 ± 2	164 ± 3	yes
final rectal temperature (°C) **	38.4 ± 0.1	38.1 ± 0.1	yes
final mean skin temperature (°C) **	35.5 ± 0.2	34.4 ± 0.3	yes
final rating of perceived exertion **	17 ± 0.6	14 ± 0.3	yes
final whole-body sweat rate (L/h)	1.0 ± 0.1	0.8 ± 0.1	no

* - or reaching prescribed safety limits of rectal temperature, heart rate, etc.
** - at same time point in both tests (i.e., the endpoint of the pre-acclimation EHT)

Each of these measurements indicate that the 12 test subjects had achieved heat acclimation at the end of the 8-week training program. Interestingly, the whole-body sweat rate was lower during the post-acclimation EHT; this likely occurred because the rectal temperature was lower (during post-acclimation testing), thereby stimulating a lower efferent sweating response at the skin.

Table 4 contains values for the following measurements taken during the EHT tests (pre- and post-training): % change in plasma volume (%CHGPV), plasma glucose, plasma lactate, plasma osmolality, and plasma cortisol. Values for pre-exercise (Pre-Ex) and post-exercise (Post-Ex) blood samples are presented. Plasma epinephrine and norepinephrine measurements are currently being performed at the Core Endocrine Laboratory of the Hershey Medical Center, Hershey, PA, under the direction of Dr. Lawrence Demers. These results will be available November 1, 1997.

TABLE 4 (All subjects combined, n = 12).

MEASUREMENT	PRE-ACCLIMATION		POST-ACCLIMATION	
	Pre-Ex	Post-Ex	Pre-Ex	Post-Ex
%CHGPV (%)		- 3.1 ± 1.2		- 4.7 ± 1.3
plasma glucose (mg/dl)	103.6 ± 4.9	107.8 ± 4.7	100.8 ± 3.7	117.4 ± 6.9
plasma lactate (mmol)	1.2 ± 0.1	2.1 ± 0.4	1.1 ± 0.1	1.7 ± 0.2
plasma osmolality	290.7 ± 0.9	295.5± 1.0	289.1 ± 0.8	298.3 ± 1.0
plasma cortisol	728 ± 63	753 ± 83	641 ± 76	849 ± 83

These measurements suggest that 8 weeks of exercise training and heat acclimation had little effect on these variables, although future statistical analyses (Years II and III) may find differences between pre- and post-acclimation cortisol concentrations.

IMMUNOLOGICAL FACTORS
The analyses performed during Year I were as follows:
1. Lymphocyte phenotypes of CD4+ (measure of T_{helper} cells) and CD8+ (measure of $T_{suppressor/cytotoxic}$ cells) by Flow Cytometry
2. Anti-Lipopolysaccharide Immunoglobulins (Anti-LPS) by ELISA
3. Total IgG by Monarch immunoassay
4. Circulating cytokines by ELISA kits.

These analyses were performed on heparinized blood samples that were collected, kept in an insulated container at room temperature and either transported immediately to USARIEM for processing for flow cytometric studies, or processed into plasma and stored at -80°C until all samples were collected and then brought frozen to USARIEM for determination of Anti-LPS, Total IgG and Cytokines. It was not possible to perform all analyses as originally proposed, because of technical/training/manpower difficulties. These

will be added during Year II and III, as described below in the section titled "Recommendations Regarding the Statement of Work".

Flow Cytometry . See **Figure 3**. The most interesting part of the data involve the very low levels of CD4+ and CD8+ present in lymphocytes, at all stages of the study. The mean values of all the subjects were outside (below) published normal ranges. These data were examined by flow cytometry experts outside USARIEM and these unexpected results were confirmed. The reason for this is unknown, but may exist because normal ranges for females of this age have not been extensively published; the ranges in Figure 3 are for groups consisting of both males and females.

Anti-LPS IgG. See **Figure 4**. From the beginning to the end of testing, the contraceptive group appeared to have greater circulating concentrations of Anti-LPS IgG than the group taking no form of contraception. Also, the combined Anti-LPS IgG tended to increase during the 8-week training program.

Total IgG. See **Figure 5**. There was no consistent trend in total IgG concentration. However, one subject (# 26) had particularly low levels of IgG (> 3 X S.D. below mean).

Cytokines: Only the results of the cytokine Tumor Necrosis Factor-a (TNF_a) have been completed (see **Figure 6,** all experimental groups combined). No changes in TNF-a and IL-10 were observed.

INF-g. See **Figure 7** (all groups experimental combined). When the concentration of INF-g was plotted against the time point in the study design, a correlation of $r = 0.99$ was observed. This trend suggests a progressive increase in INF-g during the 8-week experimental protocol, from baseline to post-training. Although not tested statistically, this trend is very interesting and deserves further analysis, in Year II and Year III. IFN-g and IL-10 are considered among the best factors to monitor the state of cytokine production because IFN-g blocks the production of the Th2 cytokines (i.e., immunostimulatory factors such as IL-4, IL-5, IL-6, IL-9, IL-10, IL-13), while IL-10 down-regulates the production of Th1 (i.e., immunosuppressive) cytokines.

Major trends were observed in several variables; these may reach significance ($p < .05$) when the number of women in the groups is increased and statistical analyses are performed (Year II and Year III). It is interesting, in this group of healthy young females, that plasma concentrations often were well below the normal range for CD4+ and CD8+ cells, and total IgG. This observation has not been previously reported in the literature and will be monitored closely during Year II and Year III.

Recommendations Regarding the Statement of Work

1. Because only one of the 12 test subjects was using Depo Provera, special efforts are currently underway to recruit Depo Provera users on the University of Connecticut campus and in the surrounding community for testing in Year II. We foresee no difficulty in securing an adequate number of eumenorrheic women or oral contraceptive users.

2. The timing of receipt of funding, ordering through the university procurement system, and delivery of two instruments (i.e., laser doppler and dew point sensor) made it impossible to use these instruments in Year I pre-training tests. Measurements of skin blood flow and local sweat rate will be made in Year II and Year III. Due to the capacities and idiosyncrasies of these instruments, pilot studies (Fall, 1997) will determine whether it will be better to measure skin blood flow and local sweat rates during EHT tests or during exercise in a cool/mild environment; either environment will provide measurements that will determine the effects of training and heat acclimation on heat dissipation and temperature regulation.

3. Similarly, because laboratory skills involving certain immunoassays (performed at USARIEM) were developed by technicians after the onset of testing, a few measurements were not possible because they must be done on the day of blood sampling. Also, two technicians learned nuances in the operation of the flow cytometer and subsequently attended a one-week intensive course on flow cytometry at Dartmouth Medical Center.

4. Future suggestions regarding analyses at USARIEM appear below:

Flow Cytometry. Technicians learned state of the art methods and preparatory techniques. As a result, they have suggested improvements in our methodologies for the following years:

a) determine additional phenotype markers (CDs) to include those associated with platelet activation and natural killer (NK) cells,

b) determine intracellular cytokines, since physical stress may increase cytokine synthesis but not their secretion,

c) develop a laboratory control from non-subjects at the University of Connecticut, and add control blood samples to those sent daily from the University of Connecticut to USARIEM.

d) we hope to develop an assay for HSP_{70} during Year II; if this is accomplished, this determination will be made on all samples.

Circulating cytokines.   Change from ELISA kits to an automated robotic system, as USARIEM's central laboratory upgrades its instrumentation.

Anti-LPS IgG.   Measure Anti-LPS in a large number of local persons to have a better laboratory control. Steps are currently being taken to accomplish these three suggestions.

5. In Year I it was necessary to transport fresh blood samples on a regular basis to both New Britain General Hospital, New Britain, CT and USARIEM, Natick, MA, because those samples could not be frozen and required timely analysis (i.e., within four hours of blood collection). Our original budget did not specify a line item for this travel expenditure, which involved nine different individuals using their privately-owned automobiles for this purpose. A review of the Project Year I budget revealed that approximately $3,000 was used for this purpose. We request the opportunity to discuss this matter with DOD in order to cover these travel expenditures during Years II and III of this project. It would be ideal to use a courier service for this purpose, to remove the burden of such travel on research personnel. We will explore the cost of a courier service prior to contacting DOD representatives.

Abbreviations Used in Figures 3 - 7

Figure 3

CD-4+ = cluster of differentiation #4; CD-8+ = cluster of differentiation #8; all subjects combined, n=12

Period 1 = Baseline I (~ 1 month pre-training)   Period 3 = Mid-training (~ 4 weeks)
Period 2 = Baseline II (pre-training)   Period 4 = After 8 weeks of training

Figure 4

LPS = lipopolysaccharide (a toxic cell wall component of gram negative bacteria)
anti-LPS = the antibody formed in response to the circulating antigen LPS
Note: All values in this figure were collected during EHT #1 and #2. The dashed line between Period 2b
    and 3 represents the boundary between pre-training (Periods 1 - 2b) and post-training (Periods 3 - 4b).
Period 1 = Pre-training (before exercise began in EHT #1)
Period 2 = Pre-training (immediately following exercise in EHT #1)
Period 2a = Pre-training (24 h after the exercise of EHT #1)
Period 2b = Pre-training (48 h after the exercise of EHT #1)
Period 3 = Post-training (before exercise began in EHT #2)
Period 4 = Post-training (immediately following exercise in EHT #2)
Period 4a = Post-training (24 h after the exercise of EHT #2)
Period 4b = Post-training (48 h after the exercise of EHT #2)

Figure 5

IgG = immunoglobulin G
birth control = eight subjects using either oral contraceptive (n = 7) or Depo Provera (n = 1) combined
no birth control = eumenorrheic ovulatory women (n = 4) who used no form of birth control
Period 1 = Pre-training (before exercise began in EHT #1)
Period 2 = Pre-training (immediately following exercise in EHT #1)
Period 3 = Pre-training (24 h after the exercise of EHT #1)
Period 4 = Pre-training (48 h after the exercise of EHT #1)
Period 5 = Post-training (before exercise began in EHT #2)
Period 6 = Post-training (immediately following exercise in EHT #2)
Period 7 = Post-training (24 h after the exercise of EHT #2)
Period 8 = Post-training (48 h after the exercise of EHT #2)

Figure 6

TNF = tumor necrosis factor (all subjects combined), a cytokine
birth control = eight subjects using either oral contraceptive (n = 7) or Depo Provera (n = 1) combined
no birth control = eumenorrheic ovulatory women (n = 4) who used no form of birth control
Period 1 = Baseline I (~ 1 month pre-training)   Period 3 = Mid-training (~ 4 weeks)
Period 2 = Baseline II (pre-training)   Period 4 = After 8 weeks of training

Figure 7

INF-g = interferon gamma
    Period 1 = Baseline I (~ 1 month pre-training)   Period 3 = Mid-training (~ 4 weeks)
    Period 2 = Baseline II (pre-training)   Period 4 = After 8 weeks of training
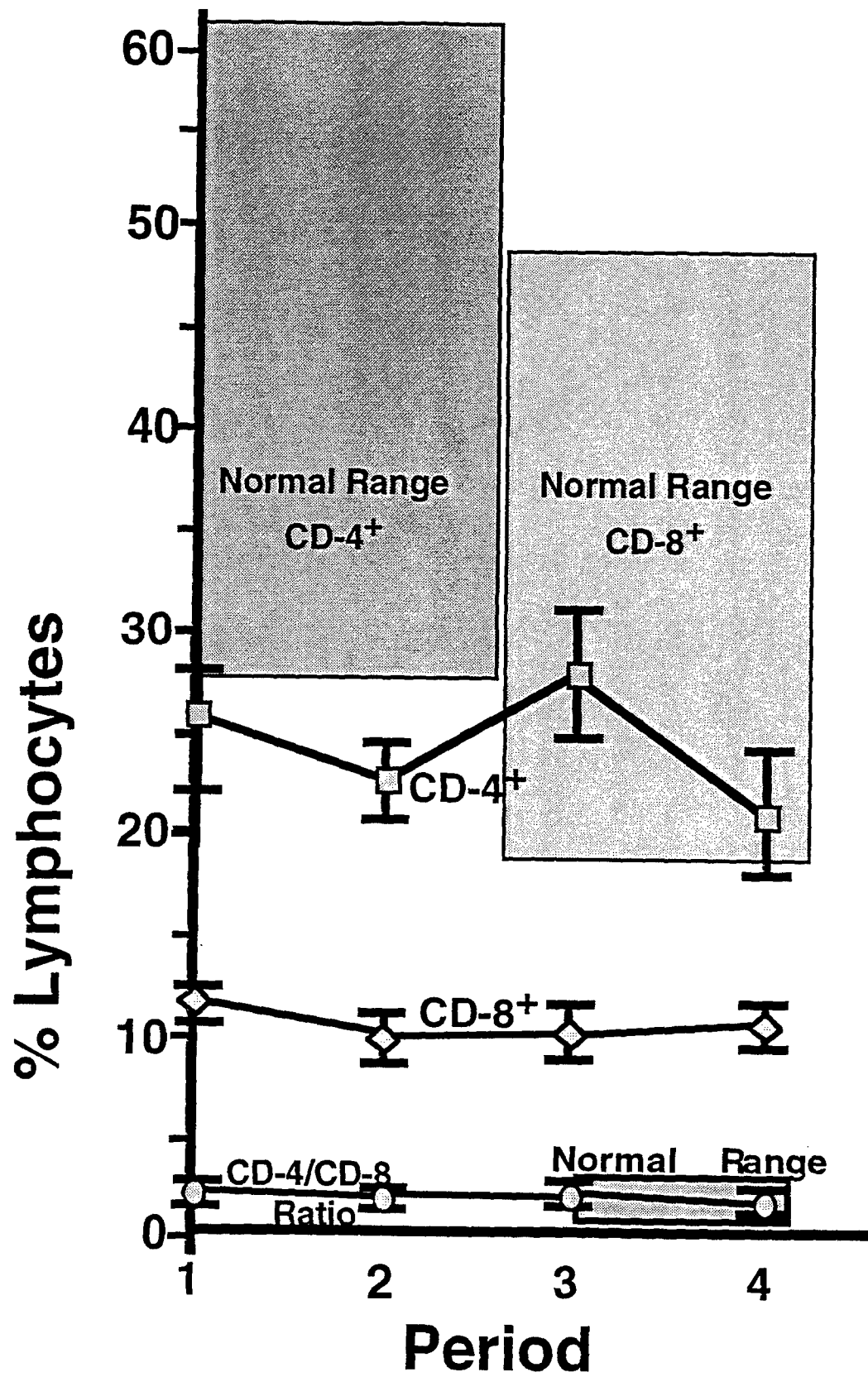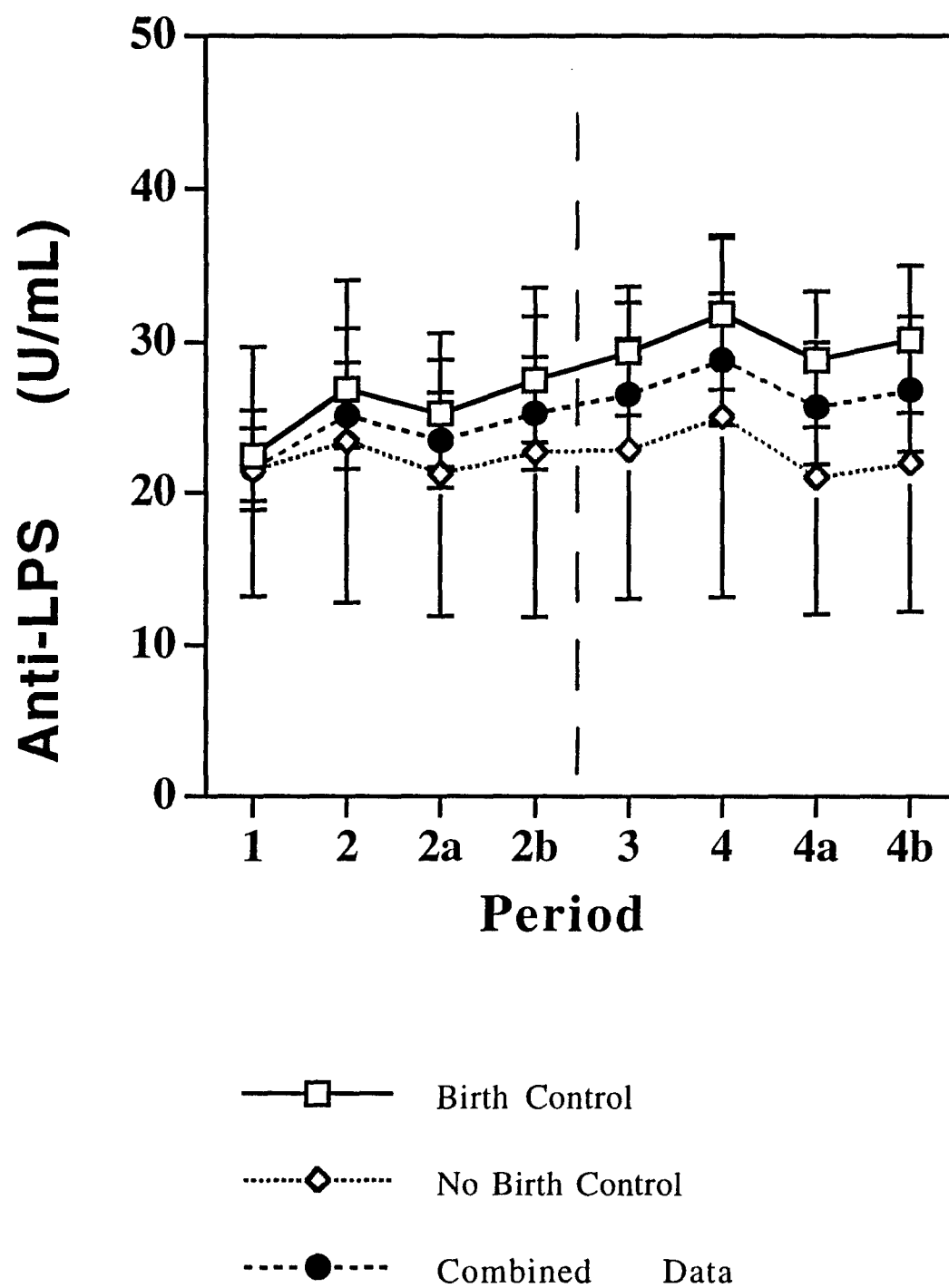
Figure 3

Figure 4

Figure 4

Figure 5

Total IgG Levels in Plasma

Legend:
- Birth Control (filled squares, solid line)
- No Birth Control (open circles, solid line)
- Combined Means (open circles, dashed line)

X-axis: Period (1–8)
Y-axis: IgG (mg/L), 500–1500

Figure 6

Figure 7

INF-g Levels in Plasma

$y = 16.95 + .423 (x)$

$r = .988$
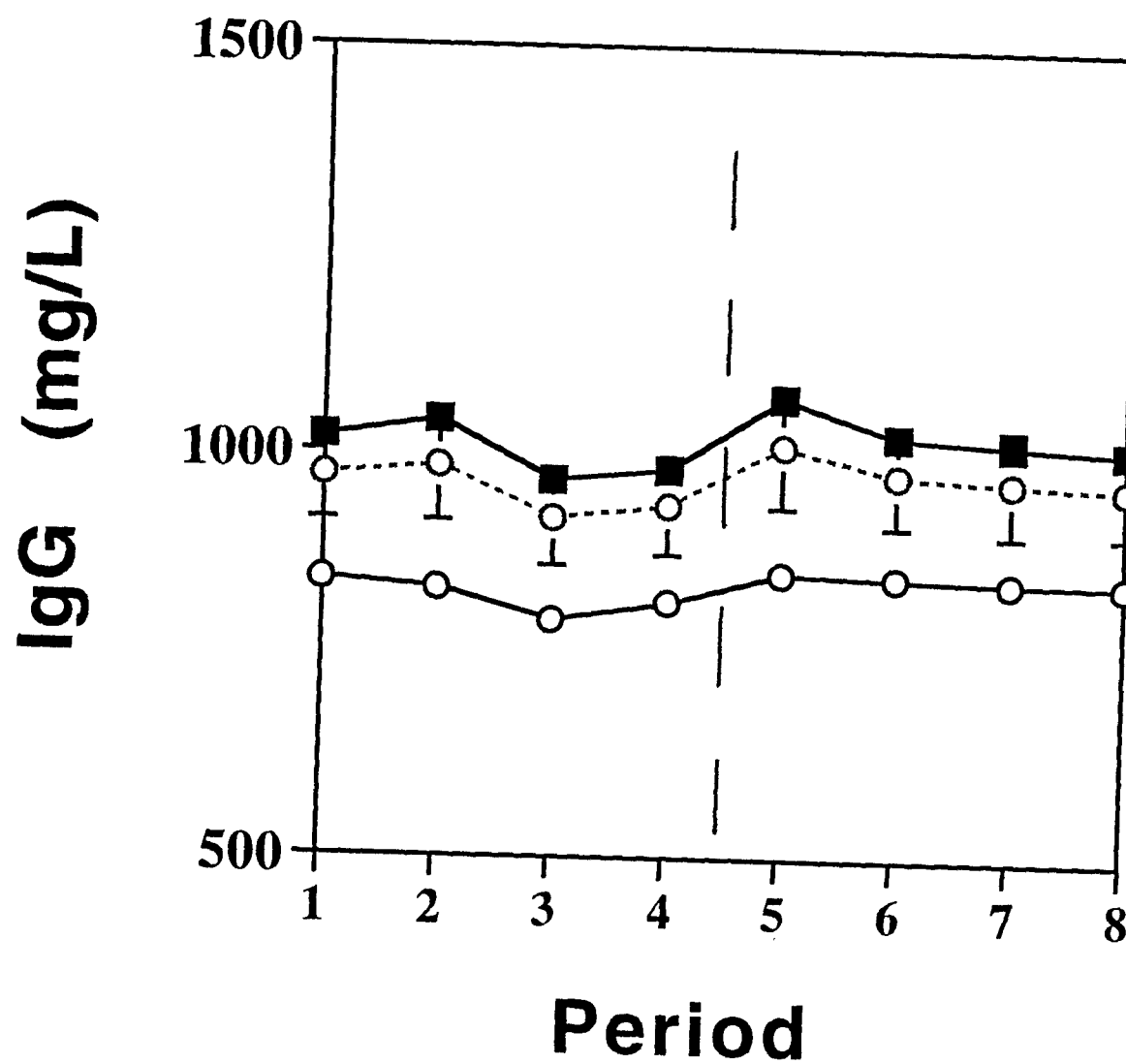
Conclusions

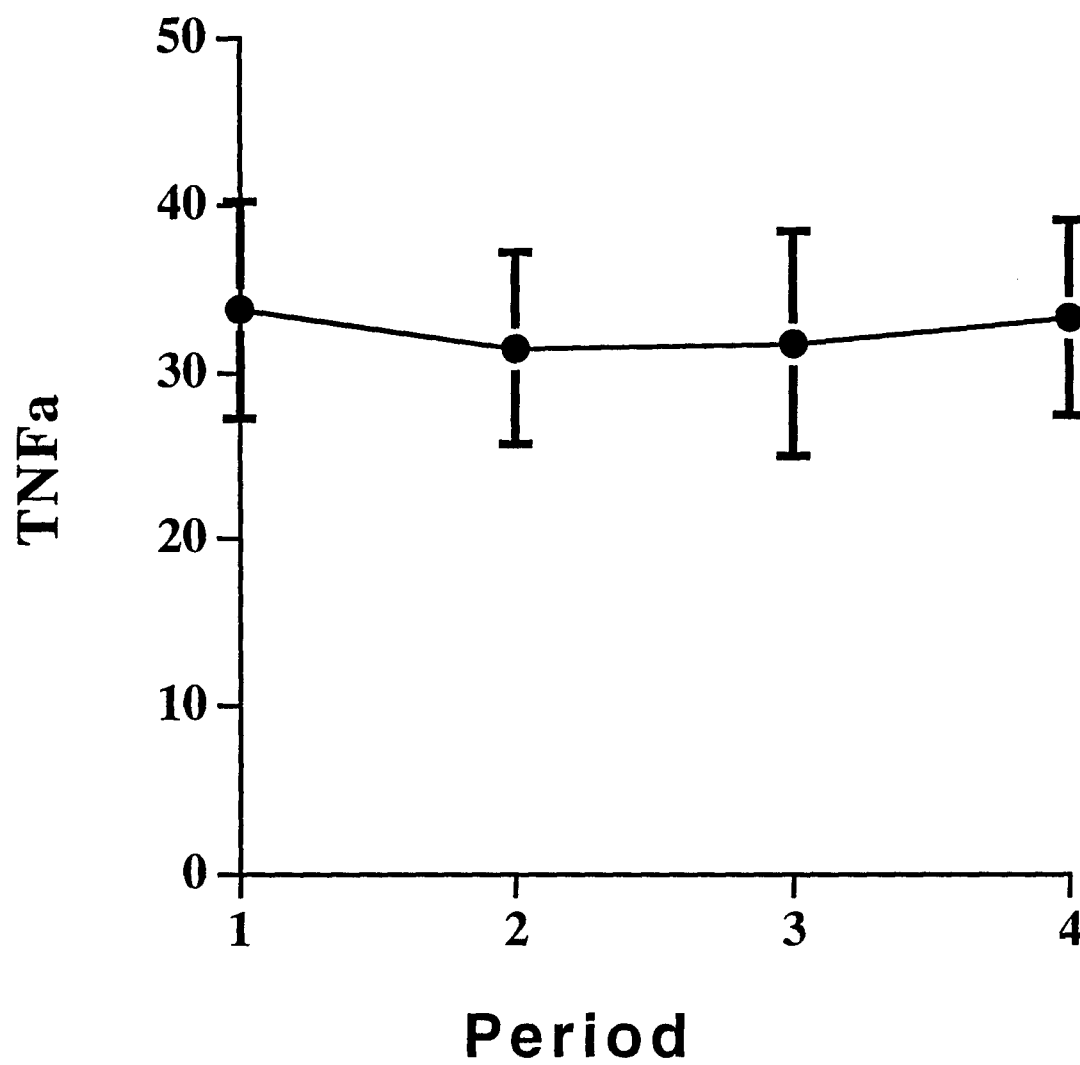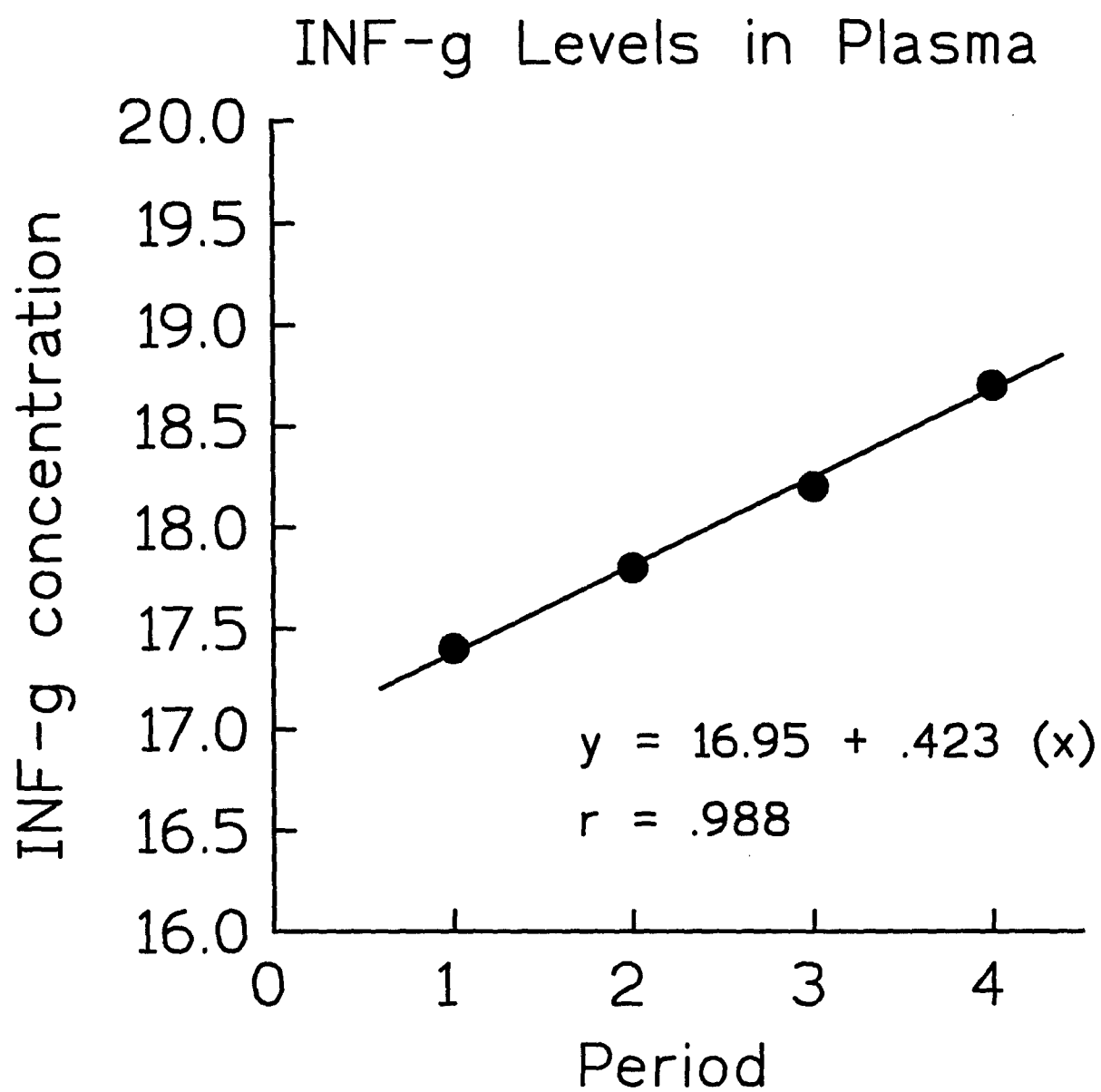1. Over 40 female volunteers were originally identified as potential test subjects, but hormonal and other physiological/clinical screening narrowed the pool to 12. These twelve test subjects completed all phases of Year I testing. Because the number of subjects was small in each treatment group (n = 1 to 7), no statistical analyses, beyond descriptive statistics, have been reported in this Year I Annual Report.

2. Each of the physical training variables indicate that the 12 test subjects were stronger, more physically fit, and leaner at the end of the 8-week physical training program.

3. Each of the variables measured during EHT tests indicate that the 12 test subjects achieved heat acclimation at the end of the 8-week training program.

4. In terms of reproductive hormone trends, study participants responded to the training regimen unremarkably. The ovulatory status of the eumenorrheic group at post-training appears similar to the pre-training period. Cycle length, follicular phase length, and luteal length appear to have remained unchanged. Hormonal responses of the contraceptive users also appear to be unremarkable.

5. The mean resting aldosterone concentrations (i.e., not associated with exercise or heat exposure) were not significantly altered by training. Future statistical analyses may reveal that there are differences in circulating cortisol levels, measured during EHT, between pre-acclimation (EHT #1) and post-acclimation (EHT #2) test sessions.

6. Immunological measures allowed the following conclusions to be drawn:
   -- there was no consistent trend in total IgG concentration
   -- the contraceptive group (both OC and DP combined) appeared to have greater circulating concentrations of Anti-LPS IgG than the EU-OV group, at all time points
   -- the contraceptive group (both OC and DP combined) tended to have greater CD-4+ levels than the EU-OV group
   -- a rising trend in INF-g was observed over time; this requires further investigation
   -- the most interesting part of the data involves the very low levels of CD4+ and CD8+, which were below the normal range; the reason for this is unknown and must be further investigated.

Individuals Who Received Funds From This Grant

	Type of Funds *
Responsible Investigators	
Lawrence E. Armstrong, Ph.D.	S, CA, ST
Carl M. Maresh, Ph.D.	S, ST
Master's Students	
Dean Aresco	1/2 GA, ST
Timothy Bilodeau	1/4 GA, ST
Jorge Herrera	1/4 GA
Sara Lozano	ST
Doctoral Students	
Tabatha Elliott	S, ST
NiCole Johnson-Keith	S, ST
Timothy Scheett	1/2 GA, S, ST
James Stoppani	1/2 GA, S, ST

* - KEY:

S, summer income

CA, conference attendance: Federation of American Societies for Experimental Biology

ST, sample transport between University of Connecticut and either USARIEM
(to Dr. Gaffin) or New Britain General Hospital (to Dr. De Souza)

GA, graduate assistantship (research assistantship) through the University of
Connecticut

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REPLY TO
ATTENTION OF:

MCMR-RMI-S   (70-1y)   23 Aug 01

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
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SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has
reexamined the need for the limitation assigned to the technical
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2. Point of contact for this request is Ms. Judy Pawlus at
DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl   PHYLIS M. RINEHART
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